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14. ABSTRACT
This project was a collaboration between two sites: The University of Missouri (MU) and the Christine M. Kleinert Institute (CMKI). The CMKI recruited and performed functional analysis and clinical sensory and motor assessments with the objective of directly comparing the overall outcomes of the hand transplant or a hand replant procedures. Participants then traveled to MU for structural and functional MRI and kinematic testing. We report six key accomplishments: 1. Functional recovery for hand transplantation is equivalent or superior to that for hand replantation. 2. Hand transplantation/ replantation is associated with substantial reductions, or elimination, of phantom limb pain and pressure. 3. The ability to localize touch without vision on the transplanted or replanted hand improves gradually over years and appears unrelated to time since amputation. 4. Development of a novel apparatus for assessment of neural activity during manual grasping in functional MRI. 5. Grasping with a transplanted or replanted hand recruits the anterior intraparietal and primary sensory cortex. 6. Individual variation in bilateral cortical reorganization of sensorimotor cortices. These have led to the development of a working model consisting of two stages of recovery following hand transplantation or replantation.

15. SUBJECT TERMS
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INTRODUCTION & BODY

Activity-dependent Cortical Organization. Primary sensory (S1) and motor (M1) cortices contain somatotopically organized maps of the body with the representation of the hand flanked laterally by the face and medially by the upper arm (*1*). These representations play a critical role in sensation and motor control, and their organization is maintained through competitive interactions that depend on sensory (afferent) and motor (efferent) activity (*2, 3*). Deafferenting injuries (e.g., limb amputation, brachial plexus lesion, or spinal cord injury) that disrupt the flow of these signals between the brain and body therefore cause pronounced cortical reorganization (*4*). These changes begin almost immediately following hand amputation, continue for months or longer, and culminate in substantial receptive field changes in the contralateral hemisphere (*5, 6*). Some S1 cortical neurons formerly responsive to stimulation of the amputated hand can subsequently be activated by stimulation of the somatotopically adjacent face and/or residual forearm (*7, 8*). Similar changes occur in M1 contralateral to the amputated hand. Years after amputation, microstimulation of M1 neurons in areas that formerly targeted amputated hand muscles can evoke movements of the residual limb or shoulder (*9*).

Despite relatively limited resolution, non-invasive studies of human amputees provide consistent evidence for gross reorganizational changes in the somatotopic organization of both S1 and M1contralateral to the amputated hand. These individuals show an expanded representation of muscles of the residual limb (*10, 11*), and in some cases a shift in the representation of muscles of the face into the former hand territory (*12*). Likewise, the sensory representation of axial body surfaces (*13*) and/or the face (*14-16*) may intrude into the former S1 hand territory. Consistent with animal models (*2*), transcranial magnetic stimulation (TMS) studies suggest that reduced levels of intrahemispheric inhibition post-injury may play a key role in these reorganizational changes (*17, 18*).

To summarize, the work described above is foundational to what has become a central tenet in modern neuroscience and subsequently a guiding principle of neurorehabilitation--that *cortical representations are activity-dependent with their organization maintained through competitive interactions.* A critical question is whether restoration of afferent and efferent activity between the hand and brain can reverse these reorganizational changes and, in doing so, yield substantial behavioral recovery.

Effects of Hand Transplantation on Cortical Organization. Recent advances in composite tissue allotransplantation (CTA) have made it possible to graft a human hand successfully. Though small in number (approximately 50 worldwide), these former amputees provide a unique opportunity to investigate whether the cortical reorganizational changes that follow amputation can be reversed. Following axotomy, the rate of peripheral nerve regeneration is affected by a variety of factors, but is estimated to proceed at approximately 2-3mm per day (*19*). This leads to gradual re-establishment of afferent and efferent signals between the transplanted hand and brain beginning proximal to the lesion and proceeding distally. However, recovery of hand function in transplant recipients is a long-term process that continues over a period of many years (*20*), which suggests a key role for experience-dependent changes in the brain.

Despite substantial variation among cases, the small number of functional neuroimaging studies of allogeneic hand transplant recipients suggest that S1 (*21, 22*) and M1 (*23-25*) can be recover at least a grossly typical map organization even when performed years or decades after the amputation. One possibility is that the changes in cortical maps that occur following amputation involve unmasking previously inhibited cortical synapses while leaving the original pattern of connectivity structurally intact (*26, 27*). Re-establishing afferent and efferent activity between the brain and hand may re-inhibit these formerly latent connections, returning the cortex to an organization similar to what was in place prior to the injury (*22*). A better understanding of these issues may have important implications not only for the success of hand transplantation, but also efforts to repair peripheral and spinal nerves and to develop neuroprosthetics.

This project was a collaboration between two sites: The University of Missouri (MU) and the Christine M. Kleinert Institute (CMKI). The CMKI recruited and performed functional analysis and clinical sensory and motor assessments with the objective of directly comparing the overall outcomes of the hand

transplant or a hand replant procedures.   Participants then traveled to MU for structural and functional MRI and kinematic testing.

KEY RESEARCH OUTCOMES

We achieved four key milestones during this award and this laid a foundation that is being built on through a newly supported CMDRP award that will enable longitudinal studies of these unique patients.

1. Functional recovery for hand transplantation is equivalent or superior to that for hand replantation.
We routinely collect functional and sensory data on our hand transplant recipients. Over the course of this study, the measurements taken have not varied significantly from data already acquired. Data was sent to the University of Missouri for correlation with the functional mapping experiments. Data from the replant subjects supported our hypothesis that functional recovery for hand transplantation was equivalent or superior to that for hand replantation. This varied between patients, with excellent functional recovery in a subject who had his hand replanted after amputation with a circular saw and poor recovery of function in hand replants following MVA with crush and avulsion injuries.

2. Hand transplantation/ replantation is associated with substantial reductions, or elimination, of phantom limb pain and pressure.
In addition to the functional data, the review of patient history and clinical course, in conjunction with the mapping studies has led to novel findings we had not previously considered. Prior to the study we had not focused on the role of transplantation or replantation on phantom pain. As data from the functional mapping demonstrated significant reorganization of the cortical maps, we re-approached all of our hand transplant recipients and asked them to grade current phantom pain. With some variation, all of our recipients reported elimination or near elimination of all types of phantom pain and pressure. Some recipients reported almost immediate relief and some subject reported resolution in months over the first year post transplant. This data is still be reviewed and correlated, but the working hypothesis is that reorganization of the cortical maps may in part result in phantom pain, and reorganization of the maps to normal geographic locations in the brain may help to resolve the symptoms, apparently permanently.

3. The ability to localize touch without vision on the transplanted or replanted hand improves gradually over years and appears unrelated to time since amputation.
As summarized in Figure 4, we find evidence for improved touch localization as a function of the amount of time that has elapsed since the procedure. Critically, the time that transplant participants spend as amputees prior to the procedure does not seem to be associated with their level of recovery.

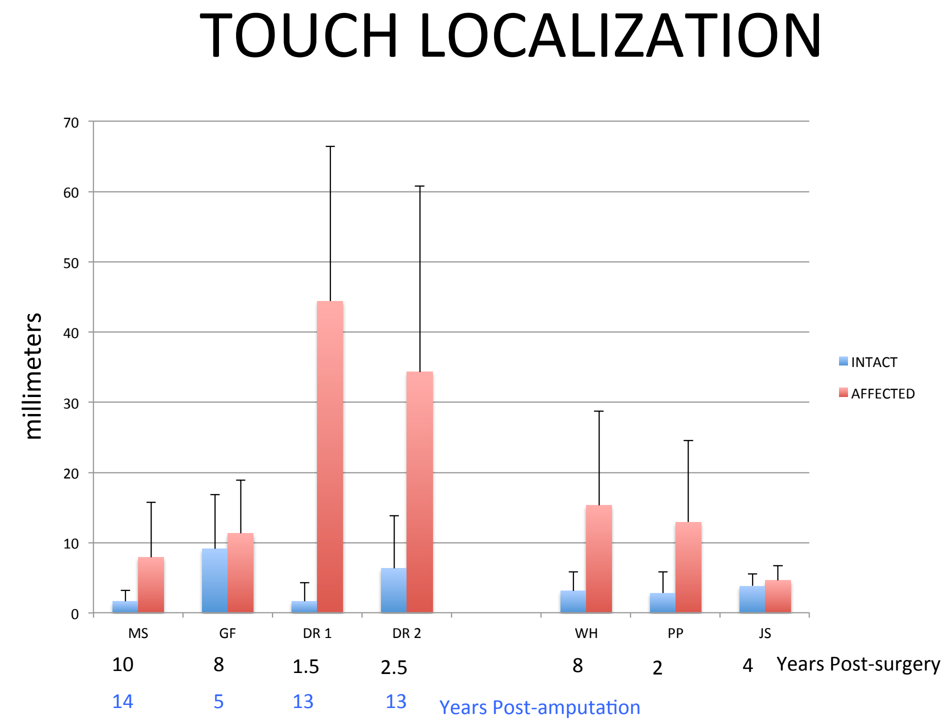

Figure 1. Accuracy of touch localization without vision of the transplanted, replanted, or intact hands. The y-axis refers to the error in millimeters. See text for details.

4. Development of a novel apparatus for assessment of neural activity during manual grasping in functional MRI.

A primary objective of the current award was to evaluate neural control of functional use of the transplanted or replanted hand. This required that we develop an MRI-compatible apparatus capable of presenting visual objects as targets for reach-to-grasp movements. After several iterations, we settled on the system illustrated in **Figure 1**. This system allows the presentation of a number of interchangeable objects. Performance is recorded with a small MRI-compatible video camera for off-line analysis. Objects can be illuminated through computer-controlled fiber-optics. Likewise, through use of a computer-controlled lighting system inside the magnet bore, we can evaluate neural activity when these actions are undertaken with and without visual feedback of the hand.

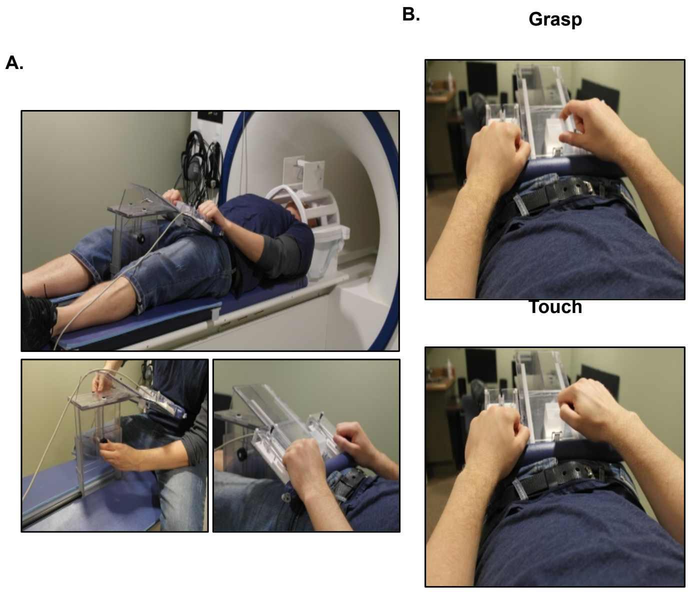

Figure 2. MRI-compatible system for investigating neural control of manual reaching and grasping movements.

5. Grasping with a transplanted or replanted hand recruits the anterior intraparietal and primary sensory cortex.

We are in the process of completing data analysis on our patients and on a larger cohort of controls. Here, we provide sample data from one hand transplant (27mths post-surgery having been an amputee for the prior 13yrs; Figure 3) and one hand replant (48mths post-surgery, immediately after amputation; Figure 4). Both participants are middle-aged males and who experienced traumatic amputations of the left distal forearm. The upper rows of each figure show differences in activity when comparing grasping vs. reaching with the affected limb. In both cases, we detect clear increases in activity within the contralateral right sensory cortex extending posteriorly into the anterior intraparietal sulcus (aIPS), a region consistently implicated in visually-guided grasping in humans and monkeys (*28, 29*). This is the first evidence that regions beyond primary sensory or motor cortex can be recruited for functional use of the hand in these patients.

6. Individual variation in bilateral cortical reorganization of sensorimotor cortices.

The lower rows in Figures 3&4 illustrate areas that exhibit increased activity during movements of the hands, face or feet. Both cases show evidence that as the sensory and motor functions of the transplanted or replanted hands recover, there is a reclaiming of the cortical hand territory. However, we also see some evidence for persistent reorganization. In the contralateral right hemisphere (Fig. 3), movements of the transplanted left hand show increased activity that extends further in the lateral direction, overlapping extensively with the face representation, a pattern consistently observed in unilateral amputees. Movements of the replanted hand (Fig. 4) show a similar effect in the contralateral hemisphere. However, this is accompanied by significant ipsilateral increases as well, which are commonly found in

unilateral amputees (*30*). These reasons for the differences are unclear but may become more clear as we complete analysis of data from our other participants.

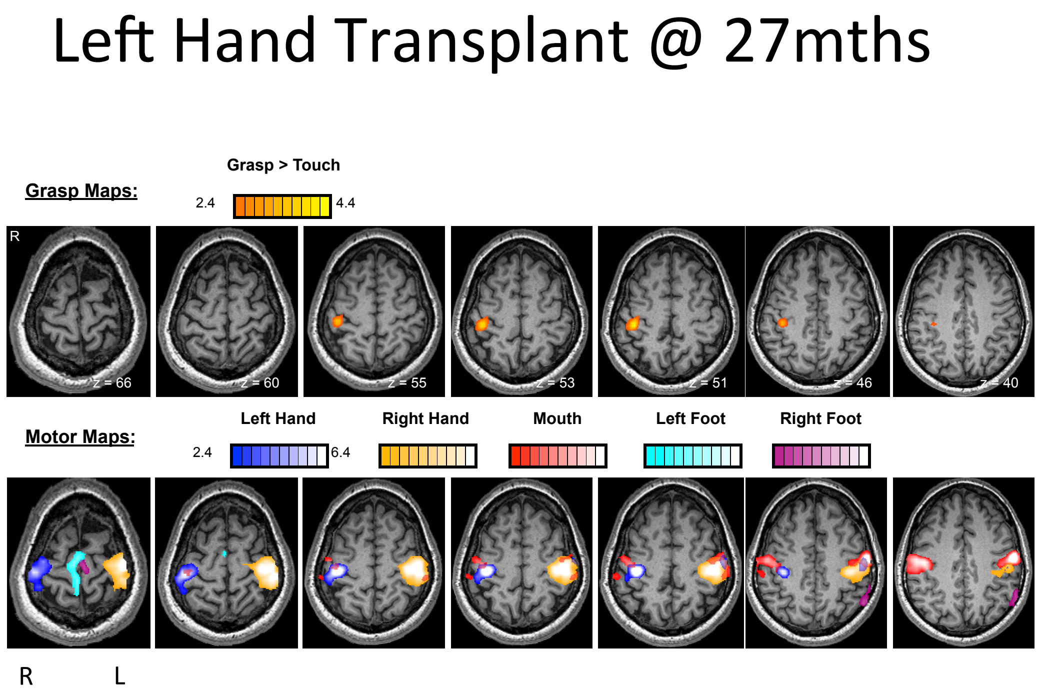

Figure 3. Functional MRI data from a unilateral left hand transplant recipient. The transplant was performed 13 years after traumatic distal forearm amputation and testing was conducted 27mths post-surgery. See text for details.

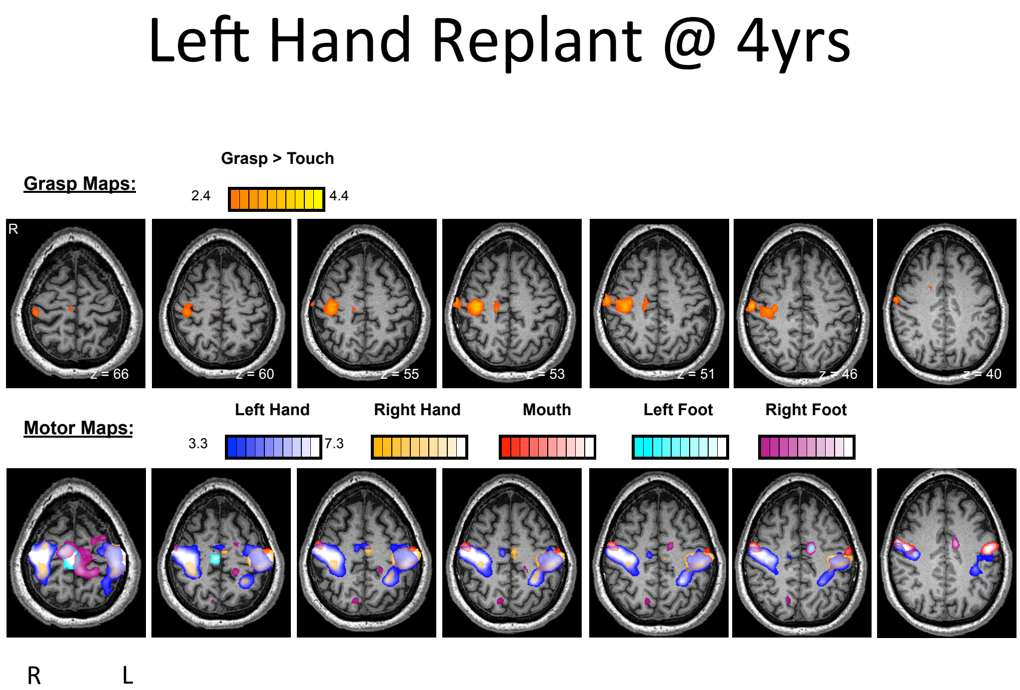

Figure 4. Functional MRI data from a unilateral left hand transplant recipient. The transplant was performed immediately after traumatic distal forearm amputation and testing was conducted 48mths post-surgery. See text for details.

6. Recovery of reach-to-grasp movement control involves a transition from an early visually-dependent state to proprioceptive control.

We used a high-resolution system to record hand kinematics when using the affected or intact hand to reach and grasp objects under two conditions: 1) Light—movements were performed with full vision of the object and hand; 2) Dark—movements were performed without vision of the hand, but the luminescent object remained visible. This latter condition forces reliance on proprioceptive control due the lack of visual feedback. Figure 5 shows data from the same hand transplant recipient illustrated earlier in Figure 3. The upper panel is data collected 15mths post-surgery and the lower panel is at 27mths. It should be noted that his “intact” hand is partially impaired. Nevertheless, at both time points, he exhibits a classic bell-shaped velocity profile when using the intact hand with clear acceleration and deceleration phases. By contrast, in the light or dark, movements of the affected hand are atypical. His peak velocity is considerably lower and much more time is spent in gradual deceleration, particularly at 15mths. Movements in the dark seem especially difficult as evidenced by lack of smooth acceleration (double peaks) at both time points when using the affected hand in the dark. When he was retested a year later, the intact side shows the same pattern. However, with the affected side, the peak velocity is higher in both conditions and deceleration is less gradual. In other words, movement control appears to become more typical, especially when vision is available.

As shown in Figure 6, after 4 years of recovery the hand replant recipient shows more typical control of the replanted hand. This suggests that both visual and proprioceptive control can be recovered and that this process may require years.

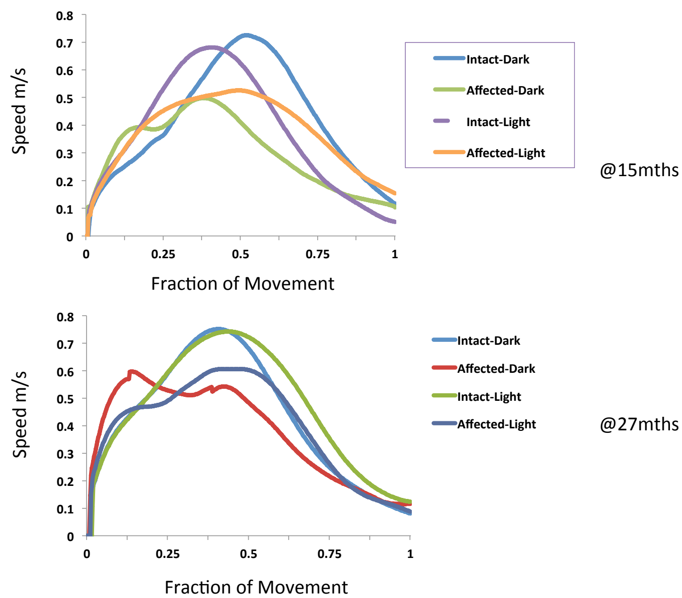

Figure 5. Kinemetics of the transplanted (affected) and intact hands during reach-to-grasp movements in with (light) and without (dark) visual feedback of the hand. Upper panel is data from 15mths after the procedure and the lower panel is from 27mths. Note that the target object is luminescent and therefor visible in all conditions. See text for details.

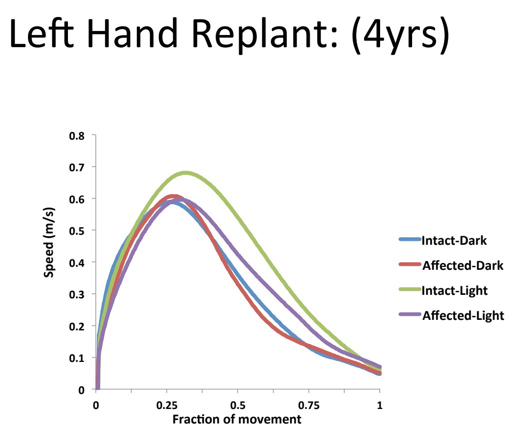

Figure 6. Kinematic data from a hand replant recipient tested 4 years post-procedure. See text for details.

Challenges of the Study

The single most challenging area of the study is subject recruitment. We have contacted colleagues at other hand transplant centers, as well as collaborating hand surgery centers that are performing replants. A review of all replants over the last 10 years from the Kleinert Kutz Hand Care center revealed over 25 hand and forearm replant recipients. However, after this amount of time the correct contact information was problematic, and of the patients we were able to contact, only four had an appropriate amputation level and were willing to travel to Louisville and then to Missouri. We will continue to reach out to other centers and approach other hand surgery and trauma facilities. In addition, moving forward we are

planning to add additional staff surgeons from CMKI and the Kleinert Kutz Hand Care center to widen the possible referrals of subjects to this study. Any assistance that could be provided from the funding agency in establishing contact with other centers would be greatly valued.

REPORTABLE OUTCOMES.

Peer-reviewed Manuscrips

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** *(in preparation). "The role of the anterior intraparietal sulcus in recovery of grasp kinematics following unilateral surgical hand replantation or transplantation."*

Baune, N., K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** *(in preparation). "Localization of touch on a transplanted or replanted hand."*

Philip, B.A., Valyear, K.F., Kaufman, C., Kutz, J., & **Frey, S.H.** *(in preparation). "Reorganization of sensory and motor cortical representations and their relationship to function in former hand amputees."*

Peer-reviewed chapter

Frey, S.H. (2013). *Why Brain Science is Essential to the Success of Hand Allotransplantation.* To appear in *The Science of Reconstructive Transplantation.* Gerard Brandacher (Ed.). Springer.

Conference presentations

Frey, S. H. *Central effects of hand amputation and transplantation*. American Academy of Hand Surgery. Las Vegas (January, 2012).

Frey, S. H. *Functional brain reorganization in current and former upper extremity amputees: Potential clinical implications*.
American Academy of Orthopedic Surgery. San Francisco (Feb., 2012).

* **Frey, S.H.** Human brain plasticity: lessons from current and former amputees. 19th Annual Prosthetics and Orthotics Conference. University of Missouri Med. School. May, 2013.

Philip BA, **Frey, S.H.** *Learning to draw with the non-dominant hand.* 23rd Annual Meeting of the Neural Control of Movement Society. Puerto Rico (April, 2013).

***Frey, S.H.** Psychomotor Keynote Address: "*Reorganization in Sensory and Motor Systems: Insights from Current and Former Hand Amputees."*
Canadian Society for Psychomotor Learning and Sport Psychology.
Kelowna, B.C. (October, 2013).

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** How does the brain support the recovery of hand function for grasping following surgical hand replantation or transplantation? Am. Soc. Of Neurorehab. San Diego (Oct, 2013).

***Frey, S.H.** "The challenge of individual variability in rehabilitation neuroscience applications of fMRI. Am. Soc. Of Neurorehab. San Diego (Oct, 2013).

CONCLUSION

We continue to work on the final data analyses and preparation of associated manuscripts. Results of our work thus far are detailed below. These have led to the development of a working model consisting of two stages of recovery following hand transplantation or replantation. This serves as the foundation on which we will build in our continued work with this unique population.

- **Stage 1: Fast functional rebalancing of existing inhibitory and excitatory synapses**
  - Synchronous with peripheral nerve regeneration (~2mm per day)
  - Coarse latent somatotopy retained because major structural connectivity is intact
  - Gross closed loop control
  - Reduction in neuropathic (phantom) limb pain.
- **Stage 2: Slow re-establishment of intrinsic hand territory**
  - Late recovering and dependent on training
  - Critical for: sensory localization, non-synergistic control of hand function, open-loop control

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APPENDICES
N/A